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# RESISTANCE DEVELOPMENT IN WHEAT SEEDLINGS AGAINST LEAD TOXICITY THROUGH *TRICHODERMA HARZIANUM* SEED COATING METHOD.

**MALEEHA UMBER, RASHIDA SULTANA, FAIZA NASIR, RAGHEEBA SAHAR AND RIZWANA MUBASHIR**

*Department of Botany, Abdus Salam School of Sciences,*
*Nusrat Jahan College Rabwah, Chenab Nagar, Pakistan.*
*Corresponding author email: maleeha.umber@njc.edu.pk*

**خلاصہ**

نصرت جہاں کالج ربوہ پاکستان کے باٹنی ڈیپارٹمنٹ میں گندم کی اقسام پنجاب 11 اور شفق 6 کے بیجوں پر ایک تجربہ کیا گیا جس کا مقصد یہ معلوم کرنا تھا کہ کیا *Trichoderma harzianum* گندم پر سیسہ کے زہریلے اثرات کے خلاف دفاعی نظام کو مضبوط کرتا ہے۔ مندرجہ بالا گندم کے بیج اور *Trichoderma harzianum* فنگس کی تہہ چڑھائی گئی اور ہوا میں خشک کر کے ریت سے بھرے کپوں میں کاشت کی گئی۔ گندم کی پنیری جب دو پتوں کی عمر تک پہنچی تب اس پر سیسہ کلورائڈ کا 3mm اور 15mm مقدار میں چھڑکاؤ کیا گیا۔ 30 دنوں کے بعد پنیری کی کٹائی کر کے اس کی جڑ اور تنے کو علیحدہ علیحدہ 50mm پوٹاشیم فاسفیٹ بفر میں محفوظ کیا گیا۔ اس کے بعد محفوظ کی گئی جڑوں اور تنوں کو پیسا گیا اور *Centrifuge* کیا گیا۔ *Centrifuge* کئے ہوئے نمونوں پر مختلف بائیو کیمیائی ٹیسٹ کئے گئے۔ ان ٹیسٹوں کے نتائج سے یہ بات امر واقع کے طور پر ثابت ہوئی ہے۔ *Trichoderma harzianum* نے گندم کی پنیری میں سیسہ کے دباؤ کے خلاف دفاعی نظام کو بہت مضبوط کیا ہے۔

## Abstract

A study was conducted to check if *Trichoderma harzianum* can develop resistance in wheat seedlings of Punjab-11 and Shafaq-06 cultivars against lead toxicity at Botany Department, Nusrat Jahan College Rabwah Pakistan. Above mentioned wheat seeds and *Trichoderma* fungus were taken from” National Agriculture Research Center” NARC Pakistan. Experiment was completely randomized design. Seeds after surface sterilization were coated with *Trichoderma* fungus and after air drying were sowed in sand cups. Lead chloride stress ( Pb1:3mM and Pb2:15mM) was given at two leaf stage of wheat seedlings. After 30 days seedlings were harvested and preserved in 50mM potassium phosphate buffer. Roots and shoots were separately preserved. Preserved roots and shoots were ground and centrifuged. Centrifuged samples were processed for various biochemical tests. Results of these tests showed great decline in hydrogen peroxide concentration and MDA content in *Trichoderma* coated seedlings as compared to non coated seedlings under lead stress. Total phenolics content and total protein content increased in *Trichoderma* treated seedlings in comparison to non treated seedlings showing eradication of harms of lead stress.

**Key words:** *Trichoderma*, lead stress, wheat, seedlings, seed coating.

## Introduction

Day to day advancing levels of industrialization and anthropogenic activities have increased heavy metal toxicity in particular lead contamination to an alarming situation harming agriculture, mankind and entire wild life (Gratao *et al*., 2005). In most regions of the world lead contamination in soil has crossed permissible limits resultantly crops grown in such soil also contain lead accumulations in them (Niragu, 1996). Lead content which is absorbed by plants is directly proportional to how much amount is present in the soil. Most readily available forms of lead in soil for plants are lead citrate, acetate, nitrate and sulfide (Lopez *et al*., 2009). Growth retardation in plants is foremost apparent symptom of lead pollution, next to this symptom is disturbance in metabolic activities (Lin *et al*., 2007). Photosynthetic pigments are also affected due to lead stress consequently damaging overall photosynthesis process (Maksymeic *et al*., 2007).

Triticum aestivum commonly called wheat is world’s most demanding crop that meets the diet requirements of thousands of people around the globe. Economics of most of the agricultural countries depend on cultivation, import and export of this wheat crop. Unfortunately wheat is also susceptible to lead toxicity. Grains of wheat grown in lead contaminated regions contain lead in them which makes them unhealthy for consumption. Lead stress can drastically harm wheat crops that will ultimately cause unbearable losses to economic conditions. Keeping in view this worth of wheat crop and alarming harms of lead stress, this current research was planned to use *Trichoderma harzianum* seed coating method on wheat seeds in order to check if resistance can be developed in this crop by above mentioned fungus treatment against lead toxicity.

*Trichoderma* is a soil born fungus that belongs to ascomycetes group and is usually identified by development of its dark green colored colonies (Druzhinina *et al.*, 2011). Various species of this fungus have been known for overcoming biotic and abiotic strains (Routtray *et al*., 2016). According to Lopez *et al*. (2015) *Trichoderma* species produce certain hormones and phenolic compounds which assist resisting a biotic stresses and enhance root system. This fungal treatment has also modified growth patterns in pepper, bottle gourd, lettuce, tomato, cucumber and reddish under abiotic stresses (Kashyap *et al*., 2017). Application of *Trichoderma* to rice crop under draught stress also functions well in reducing MDA content, hydrogen peroxide content and increasing phenolics as well as APX activity ( Shukla *et al.*,2012).

**Materials and Methods**

Initially the surface of Seeds of Punjab-11 and Shafaq-06 wheat cultivars was sterilized and than coated with *Trichoderma harzianum* at the rate of 3x $10^6$ CFU. After keeping for air drying for one day seeds were sown in sand in replicates of three sets. Control set without lead stress, NT set with lead stress but without fungal coating while Pb1 and Pb2 sets with lead chloride stress (3mM and 15mM) as well as fungal coating. Stress was applied at 2 leaf stage of wheat seedlings. Seedlings were harvested after 30 days. Roots, shoots were individually grinded and centrifuged in potassium phosphate buffer of 50 M at 14000 rpm for fifteen minutes. Than all supernatants were examined biochemically.

**MDA content**

Dhindsa *et al*., (1981) protocol was used with few changes for determining MDA content. 2ml supernatant was taken in which 2ml of 0.6% TBA was added and than heated for 20 minutes in water bath at 100 ℃ . On completing heating duration samples were instantly cooled for 20 minutes and than reading was taken at 532 nm on spectrophotometer.

**Hydrogen peroxide content**

Hydrogen peroxide content was determined using Velikova *et al*., (2000) method. 0.1 ml of supernatant were added to 0.1 ml of 10 Mm potassium phosphate buffer (PH 7.0) and 1M IKI (Iodine-potassium-iodide).The absorbance was taken 390nm.

**APX activity**

This biochemical test of measuring APX activity was done through Asada and Takahashi (1987) procedure. Reaction mixture (1600µl) had 50 mM potassium phosphate buffer, 0.5 mM ascorbic acid, 0.1 mM $H_2O_2$ and 400 µl enzyme extract. The absorbance was taken at 290 nm against the blank and the enzyme activity was represented in $Umg^{-1}$ protein (U=change in 0.1 absorbance min-1 $mg^{-1}$ protein).

**Total phenolics content**

Total phenolics content was determined through Folin-Ciocalteu protocol (Wolfe *et al*. 2003) with few modifications. Supernatants were mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were shaken for fifteen seconds and were permitted to stand for 30 min at 40 °C. Then absorbance was noted at 765 nm on spectrophotometer.

**1.1. Total soluble proteins:**

Total soluble proteins were determined using Bradford (1976) protocol with slight amendments. 1ml supernatant was reacted with 2ml Bradford Reagent and incubated for 15-20 minutes then reading was measured at 595 nm.

## Results and Discussion

### 1.1. MDA content:

**Table.1. Mean values of MDA in shoots and roots of wheat cultivars at different treatments.**

| Unit: umol/gFW | SHAFAQ | PUNJAB |
|---|---|---|
| control shoot | 0.132 | 0.127 |
| U1=untreated,no trichoderma | 0.142 | 0.167 |
| U2= untreated, no trichoderma | 0.165 | 0.172 |
| Pb1= 3mM | 0.125 | 0.124 |
| Pb2= 15mM | 0.119 | 0.106 |
| control root | 0.104 | 0.121 |
| U1=untreated,no trichoderma | 0.139 | 0.147 |
| U2= untreated, no trichoderma | 0.147 | 0.159 |
| Pb1= 3mM | 0.109 | 0.112 |
| Pb2= 15mM | 0.096 | 0.103 |

According to results depicted in Table 1 MDA content has markedly decreased in *Trichoderma* seed coated set under both levels of lead chloride stress as compared to control and non seed coated group. This suggests that *Trichoderma* has effectively reduced lead stress effects.

### 1.2. Hydrogen peroxide content:

**Table 2. Mean values of hydrogen peroxide in shoots and roots of wheat cultivars.**

| Unit: umol/gFW | SHAFAQ | PUNJAB |
|---|---|---|
| control shoot | 0.235 | 0.225 |
| U1=untreated,no trichoderma | 0.279 | 0.247 |
| U2= untreated, no trichoderma | 0.288 | 0.255 |
| Pb1= 3mM | 0.216 | 0.196 |
| Pb2= 15mM | 0.202 | 0.187 |
| control root | 0.228 | 0.23 |
| U1=untreated,no trichoderma | 0.2606 | 0.268 |
| U2= untreated, no trichoderma | 0.267 | 0.275 |
| Pb1= 3mM | 0.214 | 0.193 |
| Pb2= 15mM | 0.203 | 0.181 |

Hydrogen peroxide content has prominently reduced under both stress levels of lead chloride in *Trichoderma* treated group while in control group and non treated group such reduction was not observed (Table 2).

#### 1.2.1. APX activity:

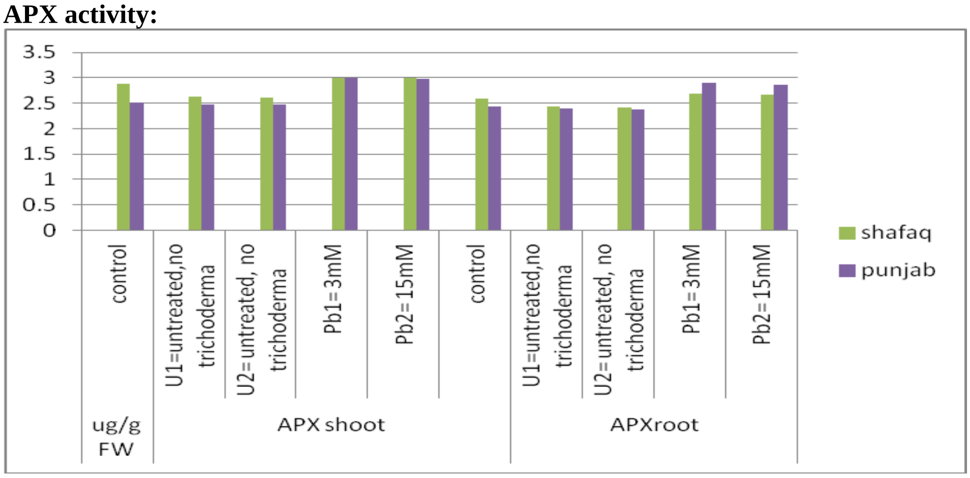


**Fig.1. Mean values of APX activity of wheat cultivars at different treatments. (X axis : replicates , Y axis: concentration)**

Results of Figure 1 show that APX activity has been increased with increase in lead stress in *Trichoderma* seed coated seedlings indicating that fungal coating has boosted defence mechanism of wheat against lead toxicity. On the other hand no such enhanced APX activity was observered in control and non coated seedlings.

**1.3. Total phenolics content:**

*Trichoderma* coated seedlings had enhanced levels of total phenolics at Pb1 and Pb2 stress levels while seedlings without fungal coating and control group seedlings were not able to raise phenolics content under stress conditions (Figure 2). Results of this test also proved that fungus has effectivly overcame lead stress.

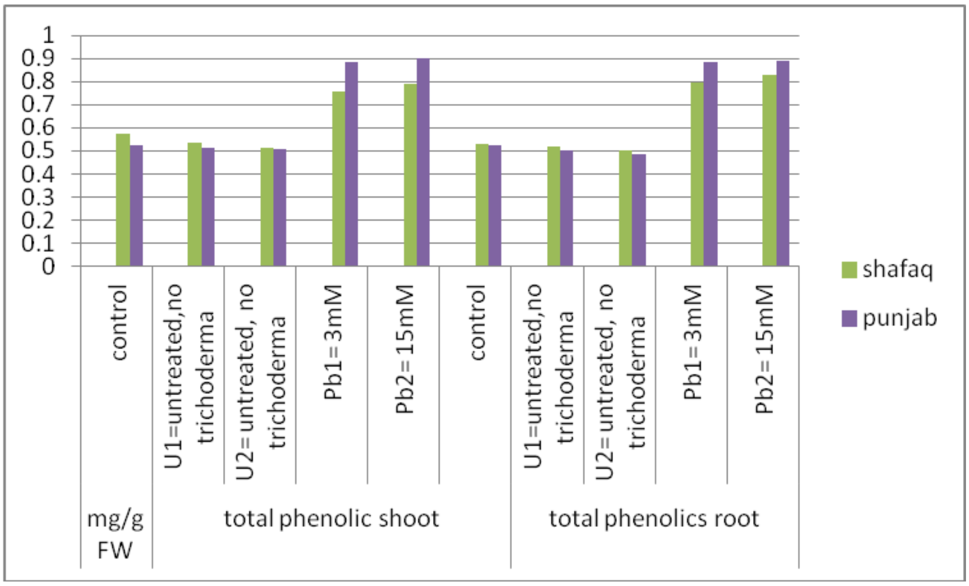


**Fig.2. Mean values of total phenolics content of wheat cultivars at different treatments. (X axis : replicates , Y axis: concentration)**

**1.4. Total soluble proteins:**

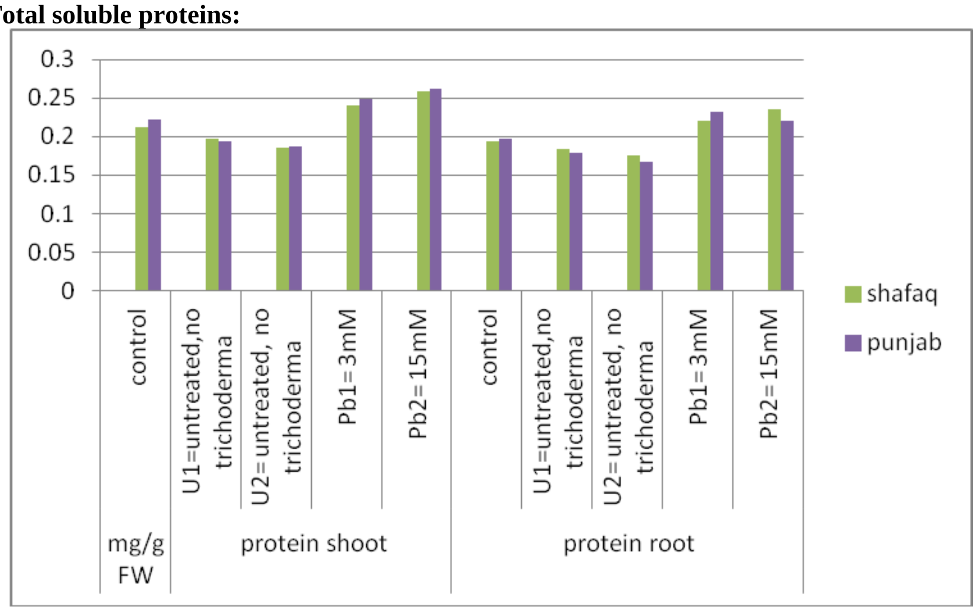


**Fig.3. Mean values of total soluble proteins of wheat cultivars at different treatments. (X axis : replicates , Y axis: concentration)**

According to figure 3 total soluble proteins were increased even under both levels of lead stress in fungal treated seedlings while non treated and control group seedlings were deprived of this advancement in protein content under stress conditions.

In accordance to all findings of our study it is very much apparent that *Trichoderma harzianum* seed coating method has been proved to be alot beneficial in developing resistance against lead chloride stress in wheat seedlings of both cultivars. Increased levels of total phenolics and APX activity proves that this fungus under lead stress of both 3mM and 15mM have boosted plant defense mechanism by triggering production of phenolic compounds and peroxidases which further stimulate production and activation of Glutathion S-transeferase like proteins which act as ROS scavengers. Due to this ROS scavenging activity MDA and hydrogen peroxide content gets reduced as was visible in our results protecting crop from harms of oxidative damages which in otherwise case are caused due to seepage of free radicals. Our work is also in accordance to previous multiple types of works such as Routray *et al.*, 2016 work with regard to *Tricoderma* as bioagent in eliminating biotic and abiotic stresses. Shukla *et al*., 2012 work shows great resemblance with the findings of this current research. Their work has shown pronounced increment of APX activity and phenolics content which decreased hydrogen peroxide concentration as well as MDA content in rice crop during draught stress through *Trichoderma* application. Similar to this, current research has also revealed that seed coating method of *Trichoderma harzianum* against lead toxicity is very effective in generating high levels of phenolic compounds and APX activity which retard ROS species like hydrogen peroxide and MDA Content. On the basis of entire working of this research paper it is strongly recommended to use *Trichoderma harzianum* seed coating method to overcome lead stress.

## Conclusion

Hence, findings of our research conclude that seed coating with *Trichoderma harzianum* prevent plants from the harms of lead stress by boosting plants defense mechanism against stress. ROS scavenging proteins like Glutathione-S –Transferase are produced due to activation of peroxidases and phenolics which are stimulated by *Trichoderma harzianum*.

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